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# Interventions to Reduce/Eliminate *Escherichia coli* O157:H7 in Ground Beef

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# Interventions to reduce/eliminate *Escherichia coli* O157:H7 in ground beef

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**Abstract**

The *Escherichia coli* O157:H7 (*E. coli* O157:H7) outbreak in the Northwestern United States ushered in an era that has dramatically changed the way beef processors in the United States convert live cattle into meat. Unprecedented cooperation among the beef processors and massive investment in research by the US government and the beef industry have resulted in an acceptable level of control of *E. coli* O157:H7 in ground beef. The evidence to support the progress in control of *E. coli* O157:H7 is the CDC data for reduction in human illness as well as the dramatic reduction in the number of *E. coli* O157:H7-positive samples in USDA-FSIS ground beef monitoring. This manuscript highlights some of the recent findings from our laboratory on the control of *E. coli* O157:H7 in ground beef. We have also summarized the key events/decisions/milestones that have contributed to the control of *E. coli* O157:H7 in ground beef in the United States. While there is much to be done to bring *E. coli* O157:H7 under complete control in the beef sector of the food industry, *E. coli* O157:H7 also is becoming a major issue in the fresh vegetable sector, as evidenced by the 2006 outbreaks in the United States. We have discussed how the fresh vegetable industry can benefit from the beef industry's experience to expedite the control of *E. coli* O157:H7 in their products.


*Keywords:* *Escherichia coli* O157:H7; Non-O157 Shiga toxin-producing *E. coli*; *Salmonella*; Interventions; Test-and-hold

## 1. Introduction

The 1993 outbreak of *Escherichia coli* O157:H7 in the Northwestern United States began an era of intense effort to eliminate this pathogen from the red meat supply. The US meat industry and government have invested millions of dollars in research leading to control of *E. coli* O157:H7. These efforts have been very successful, as demonstrated by CDC reports of *E. coli* O157:H7-related illnesses, which were 1.03, 0.9, and 1.06 per 100,000 populations for 2003, 2004, and 2005, respectively (Anonymous, 2005). The Healthy People 2010 goal is 1.0 *E. coli* O157:H7-related illness per 100,000 populations. This progress is the result of the implementation of research findings by the industry as well as a process called test-and-hold (for review, Koohmaraie et al., 2005).

In spite of all efforts and great vigilance by the beef processing sector, there continued to be ground beef-related illnesses and costly product recalls. Thus, in the late 1990s some members of the industry began to implement the test-and-hold process (Brabban, Nelsen, Kutter, Edrington, & Callaway, 2004). To minimize the likelihood of the finished product containing *E. coli* O157:H7, the raw ground beef materials (beef trim) or finished ground beef would be sampled, tested for presence of *E. coli* O157:H7 and if results were negative the product would be released into commerce. If positive, the entire lot (typically 10,000 pounds for trim and 1 h of production or about 90,000

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pounds for ground beef) would be diverted to fully cooked product, or rendered. Soon the rest of industry adopted this costly process. The test-and-hold process costs the beef processing sector millions of dollars annually.

Since the implementation of the test-and-hold process, we at the US Meat Animal Research Center (USMARC) have been conducting research to reduce the number of positive samples identified by test-and-hold, thereby increasing the safety of the food supply and decreasing the economic burden on the beef processing sector. For example, the knowledge that pathogens originate primarily from hides and that they are transferred to carcasses during the hide removal process (Barkocy-Gallagher et al., 2003; Bosilevac et al., 2004; Bosilevac, Nou, Osborn, Allen, & Koohmaraie, 2005; Bosilevac, Shackelford, Brichta, & Koohmaraie, 2005; Nou et al., 2003) has greatly helped the industry in reducing the number of positive samples identified by test-and-hold. Some processors have implemented the hide-on carcass wash intervention system, some have spent a great deal of time training their employees to properly remove hides so as to minimize transfer of pathogens from hides to carcasses, and others are attempting to implement new interventions based on this knowledge. This manuscript describes additional contributions from our laboratory aimed at further reducing *E. coli* O157:H7 from the red meat supply.

## 2. Escherichia coli O157:H7

*Escherichia coli* serotype O157:H7 and its significance to public health and commerce are well documented. This bacterium is capable of producing large quantities of toxins (Shiga toxins) that cause severe damage to the intestinal lining and is recognized as the causative agent of outbreak hemorrhagic diarrhea (Johnson, Lior, & Bezanson, 1983; Riley et al., 1983). It has been established that *E. coli* O157:H7 can be found in animals and is associated with contaminated meat (Chapman et al., 1993; Hancock, Besser, Lejeune, Davis, & Rice, 2001). For a more thorough discussion of all aspects of *E. coli* O157:H7, the reader is referred to a number of review papers that have been written on the subject, including Acheson (2000) and Koohmaraie et al. (2005).

## 3. Development of methodology to allow routine enumerations of *E. coli* O157:H7

Several years ago, we recognized the significance of enumeration data and we began to use the available enumeration method at the time, most probable number (MPN), to enumerate *E. coli* O157:H7 on hides and to quantify the effect of interventions in reducing *E. coli* O157:H7. We decided to develop alternative enumeration methodology because: (1) the MPN methodology was very expensive, costing about \$100 per sample in 2001, thereby dramatically reducing the number of samples that can be subjected to this analysis; (2) MPN results were often inconsistent, raising doubt about the validity of the results; and (3) MPN assay is labor intensive. Therefore we began work on the development of an enumeration method that does not have the problems associated with MPN assay. We now have developed and validated two high-throughput methods for the enumeration of *Salmonella* and *E. coli* O157:H7 from various sample types (feces, hides, carcass sponges, ground beef, and beef trim) collected during beef processing (Brichta-Harhay et al., 2007). These methods can be performed in a fraction of the time and cost of other culture-based enumeration methods such as MPN analyses. Consequently, their use allows for routine testing and quantification, thus providing useful information about the effectiveness of intervention strategies. The availability of accurate and inexpensive enumeration methods would also aid those involved in food safety research, as evidenced by the example given below. The power of such an assay became clear to us when we examined the efficacy of hide washing to control *E. coli* O157:H7 levels on hides (Arthur, Bosilevac, Brichta-Harhay, Kalchayanand et al., 2007). We had clearly shown that hide wash interventions were effective controls for *E. coli* O157:H7 (Bosilevac et al., 2004; Bosilevac, Nou et al., 2005; Bosilevac, Shackelford et al., 2005). The measure of effectiveness for these hide interventions was based on reductions in prevalence. Most current prevalence assays for *E. coli* O157:H7 are quite sensitive for the target organism and will give positive results even when only low levels of target cells (e.g., 10–50 CFU) are present in the sample (Barkocy-Gallagher et al., 2005). Therefore, hides harboring *E. coli* O157:H7 at 5 CFU/100 $cm^2$ and at 50,000 CFU/100 $cm^2$ will give the same positive results.

The interventions cited above were shown to be effective in reducing *E. coli* O157:H7 hide prevalence of beef cattle during processing, indicating that large reductions in the *E. coli* O157:H7 load on cattle hides were occurring (Bosilevac et al., 2004; Bosilevac, Nou et al., 2005; Bosilevac, Shackelford et al., 2005). These interventions, while being quite thorough, may not be suitable for all beef processing plants to implement, due to cost and space restrictions. Several years ago we tested the efficacy of washing cattle hides in a cabinet, using 100 to 200 ppm chlorine. Since we used the reduction in prevalence to determine efficacy and did not observe a significant decrease in *E. coli* O157:H7 prevalence, we erroneously concluded that washing cattle hides with 100–200 ppm chlorine was not a viable method of reducing *E. coli* O157:H7 on cattle hides. With the advent of the new enumeration methods, we re-examined the efficacy of this wash cabinet. This time we saw dramatic reductions in *E. coli* O157:H7 on carcasses in plants that were washing cattle hides with chlorine. Having developed an accurate and inexpensive method to enumerate *E. coli* O157:H7 and *Salmonella*, we were able to demonstrate that although use of this hide wash cabinet does not dramatically reduce hide prevalence of *E. coli* O157:H7 and *Salmonella*, it does significantly reduce the level of *E. coli* O157:H7 and *Salmonella* on the hides of beef cattle during processing (Table 1, Arthur, Bosilevac, Brichta-Harhay, Kalchayanand et al., 2007). For example, of the 288 carcasses in the study, in 250 samples *E. coli* O157:H7 levels were less than the detection limit (40 CFU/100 $cm^2$), compared to only 187 samples before the hides were washed with 100 ppm chlorine. Before washing hides with chlorine, 51 samples had >100 *E. coli* O157:H7/100 $cm^2$ and after chlorine washing only 14 samples had >100 *E. coli* O157:H7/100 $cm^2$. These results are extremely significant when one considers the following common knowledge. We believe all the interventions that are used in beef processing plants have the capacity to control a given but unknown level of *E. coli* O157:H7. As long as the load on the incoming cattle is within the capacity of these interventions, we do not expect the contamination of the resultant trim/ground beef to be at a level that can be detected in the test-and-hold process. The problem becomes very significant and costly (due to the amount of product that is rejected by the test-and-hold process) when the load on the incoming cattle exceeds the capacity of the interventions. For this reason we have always searched for interventions that would bring the levels of the incoming load in line with the capacity of the current intervention. Clearly, hide washing with 100–200 ppm chlorine is such an intervention. We have given all the details to a wash cabinet manufacturer and fully expect wider implementation of this very effective hide wash intervention to control *E. coli* O157:H7 on the hide, and thereby the carcass, and ultimately in ground beef.

We look forward to seeing the impact of widespread use of our affordable enumeration methods and, more importantly, the role they will play in further control of *E. coli* O157:H7 in red meat, particularly by the small and medium sized beef processing plants. We have also shown that our *E. coli* O157:H7 and *Salmonella* enumeration methods can be used to enumerate *E. coli* O157:H7 in compost and aged manure (unpublished data) and *Salmonella* in poultry carcass rinses (Brichta-Harhay et al., 2007).

Table 1
*E. coli* O157:H7 level and prevalence (%) before and after hide wash cabinet

| *n*[a] | Total |
|---|---|
| | 288 |
| Enumeration | |
| Before cabinet[b] | 35.1 |
| After cabinet | 13.2[d] |
| Prevalence | |
| Before cabinet[c] | 97.6 |
| After cabinet | 89.6[d] |

[a] Number of cattle hides sampled each trip.
[b] Enumeration data are presented as the percentage of total samples that were above the limit of detection for enumeration. Enumeration limit of detection for hide samples was $\geqslant$40 CFU/100 $cm^2$.
[c] Prevalence values given are the number of hide samples that were positive divided by the total number of hides sampled and expressed as percentage.
[d] Value differs significantly ($P \leqslant 0.05$) from value before hide wash cabinet.

## 4. Transportation and lairage environment effects on prevalence and levels of *E. coli* O157:H7 on hides and carcasses of beef cattle at processing

It is our assessment that if the current knowledge about the source of *E. coli* O157:H7 and how it is transferred to carcasses and ultimately to ground beef is put into practice, there is not much more that can be done to reduce the incidence of *E. coli* O157:H7 in ground beef during harvest (from the time that cattle are stunned until processing is completed). For a variety of reasons, we became interested in determining the relative role of lairage at the processing plant (from off loading of cattle until stunning) in *E. coli* O157:H7 contamination of carcasses (Arthur, Bosilevac, Brichta-Harhay, Guerini, Kalchayanand et al., 2007). During the summer of 2004 and at the conclusion of a 9-mo study of *E. coli* O157:H7 in a feedlot, we sampled the hides of 286 cattle and transported them to a commercial beef processing plant and sampled the hides again at the plant (after stunning and exsanguination). The prevalence of *E. coli* O157:H7 on hides increased from 50.3% to 94.4%, between loading onto tractor-trailers at the feedlot and before hide removal in the processing plant. Prior to transport, nine animals were found to have *E. coli* O157:H7 at high concentrations (>0.4 CFU/$cm^2$) on their hides. When sampled at the slaughter facility, the number of animals with high hide concentrations of *E. coli* O157:H7 had increased to 70. Overall, only 29% (221 of 764) of the *E. coli* O157:H7 isolates collected post-harvest were found to match pulsed field gel electrophoresis types collected prior to transport. The results suggested transport to and lairage at processing plants can lead to increases in the prevalence and levels of *E. coli* O157:H7 contamination on hides and the number of *E. coli* O157:H7 pulsed field gel electrophoresis types associated with the animals. To confirm these findings, the study was repeated. This time we took cattle from the same feedlot (known PFGE patterns) to three different commercial beef processing plants (Arthur et al., 2007b). We confirmed the results of the previous year and concluded that cattle holding areas at the processing plants are a major source of hide contamination and it is quite possible that cattle hides could be free of *E. coli* O157:H7 when leaving the feedlot and become highly contaminated with *E. coli* O157:H7 at the plant.

There are a number of major outcomes from this study. The first outcome of knowing that the plant holding area is a major source of contamination is that it removes the incentive for feedlot operators to use interventions at the feedlot (and as of now there is no effective pre-harvest or feedlot intervention) because the likelihood of their cattle becoming re-contaminated at the plant is extremely high.

The second outcome is that a given lot of cattle could become the source of another pathogen such as multi-drug resistant *Salmonella*. For example, in a plant that processes cull dairy cows as well as steers and heifers, the steers and heifers could be contaminated with MDR *Salmonella,* which is more often associated with cull cows than fed beef. Lastly is the realization that it is impossible to devise a cost-effective intervention to prevent cross contamination during holding of cattle at the plant. We are certain that such an intervention could be implemented, but we are equally certain that such an intervention would be cost prohibitive. In a recently completed study (unpublished data), hide intervention and/or sanitary hide removal were shown to negate the lairage effect.

## 5. Efficacy of pre-harvest versus post-harvest interventions

We have stated that harvest is the most logical and effective step in the beef production system at which to maximally reduce *E. coli* O157:H7 (as well as other pathogens) on cattle and, thereby, in ground beef (for details see Koohmaraie et al., 2005). This is not to say that we should not focus on pre-harvest controls. However, it is our belief that after large investments in research to control *E. coli* O157:H7 in live animals, to date we can not direct a producer or a feedlot operator to a practice to allow its control in the living animal. Some would use the highly publicized *E. coli* O157:H7 outbreaks in the United States in 2006, which were caused by *E. coli* O157:H7-contaminated spinach, and the yet-to-be-determined source(s) of the outbreaks at Taco Bell and Taco John's to make the case for the importance of pre-harvest over post-harvest control. In our assessment, the same case can be made for the post-harvest control of *E. coli* O157:H7 in vegetables. Government and industry have invested heavily in research that has led to the control of *E. coli* O157:H7 in ground beef at the processing plant and the same can and should be done to control *E. coli* O157:H7 in vegetables.

One of the main reasons for our conclusion that post-harvest is a far better control point than pre-harvest is that pre-harvest controls will have to be pathogen specific, for example, using vaccination or probiotics to control *E. coli* O157:H7 in cattle. Focusing on one pathogen in a pre-harvest setting may aid in control of that one pathogen, but there is far more than one pathogen that can cause human disease. For a variety of reasons, *E. coli* O157:H7 has been the focus. But there is no doubt that as we become efficient in controlling *E. coli* O157:H7, if our approach does not include the control of other pathogens, these other pathogens will then become the cause of human illness. Pathogen-specific interventions are not viable approaches. Other pathogens that have the potential to become of concern are non-O157 Shiga toxin-producing *E. coli* (non-O157 STEC), MDR *Salmonella,* and *Listeria monocytogenes,* to mention a few. This is contrasted with the fact that most, if not all, post-harvest interventions are effective against all pathogens and not just *E. coli* O157:H7. What is needed to control *E. coli* O157:H7 and other pathogens in fresh vegetables is a systematic study of their production and processing steps to identify a monitoring and control system for all pathogens of concern. The reader is referred to Koohmaraie et al. (2005) for more detailed reasoning of our preference for post-harvest control.

## 6. Significant events/decisions/milestones that have contributed to the control of *E. coli* O157:H7 in ground beef in the United States

In Table 2, we listed key events that have played a pivotal role in the control of *E. coli* O157:H7 in the US meat supply. Though close, these events are not listed in chronological order. Each of these events has contributed greatly to the collective success of all segments of the beef industry's control of *E. coli* O157:H7 in our red meat supply (Table 3), but in our assessment knowledge sharing has played the most significant role (Table 2 items #5, 6, 8, 11, and 14). Perhaps the most significant of these items with respect to knowledge sharing is the decision by the presidents of the beef processing companies to allow sharing of information and not to keep such knowledge as trade secrets. As a result, organizations such as the National Cattlemen's Beef Association can take the lead in forming the Beef Industry Food Safety Council (BIFSCo) or the Beef Safety Summits to bring the industry leaders, people that operate beef processing plants, and the scientists engaged in food safety research together to address problems. The reader is s encouraged to go to the BIFSCo website (www.BIFSCo.org). The American Meat Institute Foundation holds "Best Practices Workshops" for similar purposes, although these are more directed to employees who are in charge of operating beef processing plants.

The results presented in Fig. 1 were collected by USMARC scientists and represent one of many such examples of knowledge-sharing within the industry. The decision to conduct this study was made at a Beef Safety Summit and was carried out at the request of the participating plants and for the purpose of bench-marking. Bench-marking is routinely used to improve the process quality by all plants. Results in Fig. 1 were collected from two different beef processing plants. Since we had established that hide is the major source of *E. coli* O157:H7 and that the processes involved in the removal of the hide determine how much *E. coli* O157:H7 is transferred to the carcass, we sampled the hides (to determine the bacterial load as presented for slaughter) and then sampled the carcasses right after hide removal and before any intervention (to determine the extent of bacterial transfer from the hide onto the carcass). Such data demonstrate which plant has the lowest rate of transfer and by learning how they achieve the low rate and sharing that information with the others, they would all improve.

Table 2
List of significant events/decisions/milestones that have contributed to the control of *E. coli* O157:H7 in ground beef in United States

| | |
|---|---|
| 1 | Jack-in-the-Box outbreak in Northwestern United States (1993). |
| 2 | Industry begins the very first screening for *E. coli* O157:H7 in raw materials and finished products on a very limited basis (1994). |
| 3 | FSIS begins rigorous enforcement of zero tolerance (1994). |
| 4 | Mike Taylor (FSIS Administrator) declares *E. coli* O157:H7 to be an adulterant in ground beef in 1994 and five years later includes beef trim as well (1994). |
| 5 | Ben Nelson (Nebraska Governor) convenes the "Governor's Conference." (1995) |
| 6 | The National Livestock and Meat Board creates the "Blue Ribbon Task Force on O157:H7" (1994)". |
| 7 | USDA mandates the implementation of HACCP (1996). |
| 8 | Beef Industry Food Safety Council (BIFSCo) is formed to bring industry together to develop unified plans to control *Escherichia coli* O157:H7 in red meat (1997). |
| 9 | Availability of rapid tests for *Escherichia coli* O157:H7 detection (begins in 1993; more widespread adoption in 1998). |
| 10 | Implementation of acid, hot water, steam vacuum and other efficacious interventions (beginning in 1994 and continuing to date). |
| 11 | The Presidents of the major companies of the beef processing segment agree that food safety is a non-competitive area and encourage collaboration and knowledge sharing (1998). |
| 12 | Implementation of test-and-hold (beginning in 1995). |
| 13 | Industry searches for and implements additional interventions to reduce product loss due to test-and-hold. This process continues to date (continuous). |
| 14 | BIFSCo organizes the first *E. coli* O157:H7 summit involving all segments of the beef industry (2003). |
| 15 | Development of "Best Practices" by BIFSCO, American Meat Institute and National Meat Association (1998). |
| 16 | The scientists at the US Meat Animal Research Center demonstrate that hide is the source of *Escherichia coli* O157:H7 in red meat and develop hide wash intervention (2002). |
| 17 | Beef processors install hide-on carcass wash cabinets or intensive training of the employees to remove hide properly to minimize transfer of pathogens from hide onto the carcass (2003). |
| 18 | Elsa Murano (USDA Under Secretary) announces policy that government would not challenge a company's negative *E. coli* O157:H7 results, even if there are positives for the same production day, provided the company conducts 100% testing of materials for raw comminuted products (2002). |

Table 3
US Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS)–Microbiological results of raw ground beef products analyzed for *E. coli* O157:H7

| Year | No. of positives | No. tested | % Positive |
|---|---|---|---|
| 1994 | 0 | 891 | 0.0 |
| 1995 | 3 | 5407 | 0.05 |
| 1996 | 4 | 5703 | 0.07 |
| 1997 | 4 | 6065 | 0.07 |
| 1998 | 14[a] | 8080 | 0.17 |
| 1999 | 32[b] | 7785 | 0.4 |
| 2000 | 55 | 6375 | 0.86 |
| 2001 | 59 | 7010 | 0.84 |
| 2002 | 55 | 7025 | 0.78 |
| 2003 | 20 | 6584 | 0.30 |
| 2004 | 14 | 8010 | 0.17 |
| 2005 | 19 | 10976 | 0.17 |
| 2006 | 20 | 11779 | 0.17 |

[a] During October 1997, the amount analyzed was increased from a 25-g sample to a 325-g sample to provide increased detection sensitivity.
[b] On September 3, 1999, a new selection and detection method was introduced to further increase test sensitivity.

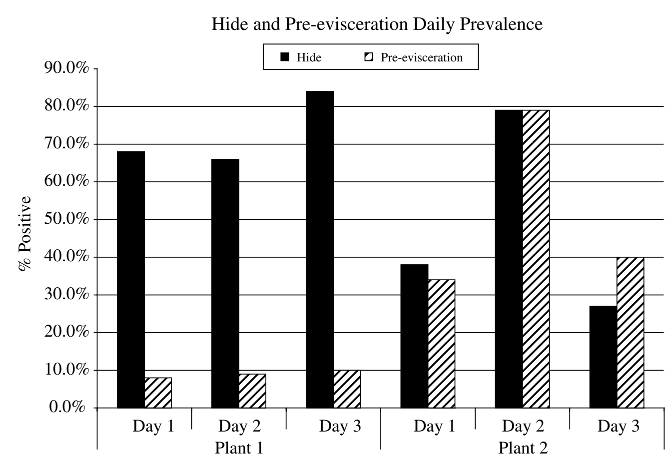


Fig. 1. The prevalence of *E. coli* O157:H7 on hides and carcasses right after hide removal in two beef processing plants. Plants were sampled on three consecutive days. Total number of samples per plant was 300 each of hide and carcass samples.

## 7. Other pathogens of concern

We believe that it is in the best interests of food-related industries to anticipate the emerging pathogens and do all of the necessary work required for their control prior to their becoming an issue. Simply put, this means industry needs to be proactive rather than reactive. *Salmonella* in general and MDR *Salmonella* specifically, non-O157 Shiga toxin-producing *E. coli* (Non-O157 STEC), *Clostridium difficile,* and *Mycobacterium avium* Paratuberculosis (MAP) are some of the organisms that are being studied. The objective is to learn as much as possible about these and other organisms that can cause human illness and develop effective controls before they become the cause of human illness and regulation is forced as a result. Clearly, if those involved in the fresh produce industry had taken this approach, they would not have experienced the devastating economic losses and been faced with the regulations that have/will follow these events.

## 8. Efficacy of post-harvest interventions against current and emerging pathogens

As discussed above, we prefer post-harvest interventions over pre-harvest interventions and have provided some of the reasoning for such a preference. Another reason is the universality of the post-harvest interventions as opposed to specificity of pre-harvest interventions. Although the notion stated in the previous sentence seems logical (i.e., heat kills all bacteria but vaccination would be bacterial specific), at the request of the industry we conducted an experiment to confirm the concept (Arthur et al., 2007a). Beef surface tissues were inoculated with various organisms, and after allowing an appropriate amount of time for attachment to take place, as well as simulating the length of time a bacterium is exposed to the carcass surface in a commercial beef processing plant before any interventions, the beef surface tissue was subjected to a number of interventions for a period of time equivalent to that used in commercial beef processing plants. The organisms used included generic *Salmonella* (Newport and Typhimurium), MDR*Salmonella* (Newport and Typhimurium), and various *E. coli* O157:H7 isolates with variations with respect to the ability of each to cause human disease. We used a number of interventions that are currently used in industry (hot water, organic acids) and some that are not currently used in industry (electrolyzed water, ozone, and Fresh FX). The results indicated that MDR *Salmonella* is reduced as effectively as *E. coli* O157:H7 when treated with antimicrobial interventions currently in use at most US beef processing plants (Arthur et al., 2007a).

## 9. Summary and conclusions

The outbreak of *E. coli* O157:H7 associated with the consumption of undercooked hamburgers in the Northwestern United States in 1992 and 1993 was the event that forced the government and the industry to control this pathogen in the US ground beef supply. The CDC data indicates that, for all practical purposes, as a nation we have met our objective (Healthy People 2010) to have only one case of *E. coli* O157:H7-related foodborne illness per 100,000 populations. We have outlined the key events that led to this success and have suggested that the principal reason for the control of *E. coli* O157:H7 in ground beef has been the sharing of knowledge obtained by massive investment in research by the government and the meat industry. Such efforts continue. This collaborative effort should be used as a model for other sectors of the food industry to control whatever issue is facing that particular sector.

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